Chapter 1

Introduction

1.1 The anomalous properties of water

Water is a unique liquid, not only because of its vital importance for life on planet earth but also because it has many exceptional properties.[1,2] In spite of its small mass and molecular volume, water has a relatively high melting and boiling point resulting from a high cohesive energy density due to its 3-dimensional hydrogen bond network. Moreover, water has an extremely low isothermal compressibility and is a poor solvent for apolar compounds. The latter property of water has led to the term "hydrophobicity", although this description might seem somewhat deceptive since London dispersion interactions between water and apolar solutes are favorable and quite marked.[3] Hydrophobic effects play an important role in many (bio)chemical processes in aqueous solution like protein folding, molecular recognition processes, aggregation of amphiphilic molecules and surface forces.[2] In studies on hydrophobic effects, two phenomena are usually distinguished: hydrophobic hydration and hydrophobic interactions.[2]

1.1.1 Hydrophobic hydration

The term hydrophobic hydration refers to the interactions of apolar solutes and water, i.e., how an apolar solute affects the water structure in its immediate environment.[2] Introduction of an apolar solute into aqueous solution is characterized by an unfavorable change in standard Gibbs energy at room temperature.[4,5,6] However, the standard enthalpy of the solution process is usually small and favorable whereas the entropy change is large and negative.[2,4,5,6] At higher temperatures, the magnitude of the standard enthalpy of solution becomes smaller and it even becomes positive at elevated temperatures. This results from the disruption of the hydrogen bond network of water at higher temperatures. Moreover, water loses its favorable interactions with the apolar solute. The large positive standard heat capacity of solution is characteristic for hydrophobic hydration.[7] The standard entropy of solution becomes less unfavorable at higher temperatures. The standard enthalpy and entropy of solution largely compensate each other resulting in a standard Gibbs energy of dissolution that is only little temperature dependent.[8]

Previously, the entropy penalty upon solution of apolar compounds in water was explained by Frank and Evans[9] in 1945 using the so-called iceberg model. Water in the hydrophobic hydration shell was stated to undergo structural enhancement (either stronger or more hydrogen bonds per volume unit) in comparison to that in the bulk, which would

account for the entropy loss and favorable enthalpy change upon solution. The enthalpy gain was accounted for by increased water-water hydrogen bonding.

Since 1945, many publications have discussed hydrophobic effects. Opinions have continually changed.[2] The current view is that water in hydrophobic hydration shells is *not* significantly more structured than that in the bulk.[2,6,10,11,12] However, recent studies indicate a strengthening of hydrogen bonds in the hydration shell of apolar solutes at room temperature whereas the reverse is observed at higher temperatures.[13] It is nevertheless widely accepted that water largely retains its original structure by accommodating the nonpolar solute in its hydrogen bonding network thereby maintaining as many hydrogen bonds as possible.[2,14] Evidence for these ideas stems from both computational[12,14,15,16] studies and experimental investigations like NMR,[17] neutron diffraction,[11] and X-ray scattering.[18] Various studies show that one of the water O-H bonds preferentially lies parallel to the nonpolar surface of the solute whereas the other bond points into bulk water.[11,15] The favorable London dispersion interactions between water and solute molecules account for the enthalpy gain upon solution. The loss of translational and rotational degrees of freedom of the water molecules in the hydrophobic hydration shell[17] is primarily responsible for the loss of entropy upon dissolving apolar compounds in water.

Indeed, insertion of a nonpolar particle in aqueous solution can be treated as two processes: formation of a cavity in the aqueous solution and onset of interactions of a solute with solvent molecules.[2] Creation of the cavity restricts the motions of solvent molecules in the hydration shell of a nonpolar solute. This restriction leads to loss of entropy, which is exceptionally large in aqueous solution due to the small size of water molecules.[19,20] Many studies have shown that solute size is the determining factor contributing to unfavorable entropy (and consequently Gibbs energy) of the solution process.[21] Onset of solute-solvent interactions is less important in determining the magnitude of the Gibbs energy of solution. Graziano studied the effect of solute size on the thermodynamic parameters of the solution of apolar solutes in aqueous solution.[22] According to this study, the work of cavity creation dominates the unfavorable Gibbs energy of solution of apolar compounds in aqueous solution. However, a distinction is made between a series of noble gases and aliphatic hydrocarbons. Solute-solvent interactions increase more rapidly than the work of cavity creation upon increasing the hard-sphere diameter of noble gases. Favorable solute-water interactions are attributed to the increase in polarizability of noble gases upon increasing their size. The polarizability effect for aliphatic hydrocarbons is less important and the decrease in solubility is caused by the dominating effect of cavity creation.

1.1.2 Hydrophobic interactions

The term “hydrophobic interactions” refers to the tendency of apolar molecules to stick together in aqueous solution.[2] These interactions play an important role in many biochemical processes like protein folding and the formation of lipid membranes. Kauzmann introduced the concept of hydrophobic interactions in 1959.[23] He explained the

entropy gain upon interaction of apolar compounds by the release of structured water molecules by destructive overlap of hydrophobic hydration shells.

Hydrophobic interactions have been classified into pairwise and bulk interactions. Pairwise hydrophobic interactions are 1:1 interactions between individually hydrated nonpolar particles in aqueous solution where the solute concentration is below the so-called critical aggregation concentration. At these concentrations aggregation of solutes is hampered by the surrounding hydration shell; actually, "apolar solutes are screened when compared to the gas phase".[2] In aqueous solution, pairwise hydrophobic interactions occur via "hydrophobic encounters" indicating that the molecules only associate transiently. Equilibrium constants for the formation of these very short-lived encounter complexes are usually smaller than unity. Nevertheless, formation of encounter complexes can account for the rate retardation effects excerted by inert cosolutes on the hydrolysis of activated esters and amides.[24] An MD simulation on the association of methane molecules in water showed that aggregation of two solute molecules is favored by entropy whereas the Gibbs energy of aggregation is slightly positive.[25]

Premicellar aggregation occurs at concentrations far below the critical aggregation concentration of the individual solutes.[26] Bulky hydrophobic quaternary ammonium halides have a strong tendency to form premicellar aggregates. These salts increase the rate of a number of chemical reactions in aqueous solution in a very efficient way.[27] Usually, the rate enhancement is larger than in the presence of micelles. Ion-pairs are formed when both components have opposite charges. The driving force for the formation of ion-pairs is both hydrophobic and electrostatic in origin.[26] Although premicellar aggregates are formed at low solute concentrations in aqueous solution, the aggregation process is considered as an example of a bulk aggregation process.

Binding of substrate molecules in binding pockets of enzymes is a 1:1 interaction process.[28] However, unlike encounter complexes, enzyme-substrate complexes exist for a longer time. Moreover, (partial) dehydration of substituents in an active site and of substrates occurs upon binding. Molecular recognition processes in biochemistry as well as in supramolecular processes strongly rely on hydrophobic interactions, although electrostatic interactions are also important.[29] Stabilization of host-guest complexes can also be increased by π-π stacking[30] and hydrogen bonding interactions.[29] Thermodynamic parameters of binding processes have been determined. A large and positive heat capacity upon complexation indicates the importance of hydrophobic interactions.[31]

Bulk hydrophobic interactions also play a decisive role in the formation of surfactant aggregates like micelles or vesicles that occurs above the critical aggregation concentration or solubility limit. Characteristic for the formation of these types of aggregates is the highly cooperative nature of the association process. Traditionally, aggregation of apolar compounds in aqueous solution above a certain critical concentration was explained by overlap of hydration shells. At a certain solute concentration, insufficient water molecules are available to form independent hydration shells and consequently water molecules have to be part of two hydration shells simultaneously, which is highly unfavorable. Upon

aggregation, part of the water molecules surrounding the individual solutes is released. This accounts for the entropy gain upon aggregation.

According to this theory of hydrophobic interactions, hydration shells of apolar solutes have to be quite extensive. However, on the basis of neutron scattering studies it was shown that hydration shells are relatively small. For example, the hydration shell of a methane molecule in aqueous solution contains ca. 19 water molecules.[32] Therefore, it has been argued that bulk hydrophobic interactions can be viewed as a special type of phase separation.[33,34] However, a final phase separated state is never reached in the case of surfactant aggregation. The process rather stops at an intermediate stage (e.g. micelles) because of favorable interactions between water molecules and surfactant head groups that prevent the formation of large structures, which would eventually lead to "real" phase separation. Phase separation occurs above the solubility limit of apolar solutes and only takes place when the mixing entropy of the solute is not sufficient to compensate for the loss of entropy of water molecules entering the hydration shells of apolar solute molecules.

1.1.3 Increasing the stabilization of hydrophobic aggregates

Protein folding is probably one of the most intriguing processes in nature in which hydrophobic interactions play a dominant role.[35,36] In addition, hydrogen bonding interactions between peptide groups, electrostatic interactions between charged amino acid residues, and London dispersion interactions contribute to the stability of the native protein structure. The main destabilizing factor in protein folding is conformational entropy, which is considerably reduced upon going from the unfolded to the folded state.[35] Participation of hydrogen bonding interactions in protein folding is beyond doubt, but their magnitude is difficult to assess. Similarly, electrostatic interactions evidently play a role although their importance is debated. Several authors emphasize the role of electrostatic interactions in protein structure and functions[37] whereas others are less convinced of their importance.[35] Ion-pairing has been shown to contribute to the Gibbs energy of proteins in their native state, which is, typically, only 20-50 kJ mol^{-1} less than their unfolded state.[36] Recently, it was suggested that ionic interactions have a stabilizing effect on the folded state. Nevertheless, these interactions also increase the stability of the unfolded state and therefore the net stability increase of the native state of the protein is relatively small.[38]

A combination of electrostatic and hydrophobic interactions is important in many other processes in (bio)chemistry. For example, aggregates consisting of oppositely charged surfactants in aqueous solution are formed at much lower concentrations than aggregates formed either from the individual components or from the components lacking the ionic charges.[39] Moreover, the morphology of aggregates differs from that of the separate surfactants. The properties of mixtures of cationic and anionic surfactants are discussed in more detail in sections 1.2.5 and 6.1.

Another example of the stabilization of two-component aggregates by hydrophobic and electrostatic interactions emerges in the hydrolysis of a cationic long-chain ester and amide by sodium hexadecanoate at low concentrations.[40] Ion-pairs are well known to be

formed at concentrations far below the critical aggregation concentration of both components and the aggregates are only small. The driving force for this type of aggregation is similar to that of micellization.[26]

Aggregation of ionic surfactants and dyes also occurs at very low concentrations. The presence of a chromophore in the dye allows the aggregation process to be followed by spectroscopic methods.[41,42,43,44] Interestingly, interactions are only observed when the charges of surfactant and dye are opposite.

These examples indicate that electrostatic interactions are an important additional factor that contribute to the stabilization of aggregates formed by hydrophobic solutes.

1.1.4 Introducing polar functionalities

Bio(chemical) molecules contain both apolar and polar functionalities. Much simpler compounds like surfactants, alcohols, or amines also consist of multiple functionalities. Introduction of polar functionalities leads to the interference of hydrophobic and hydrophilic hydration shells. A polar group reduces the overall hydrophobicity of an apolar solute by an amount equivalent to several methylene groups: the hydration shells of polar groups overlap with those of methylene groups. Alternatively, introduction of polar functionalities increases the solubility of apolar solutes since it reduces the necessity to form a complete hydrophobic hydration shell. Modification of the hydrophobic hydration shell by the hydration shell of hydrophilic groups in close proximity has been assessed in detail by studying kinetic solvent effects of added solutes on the hydrolysis of activated amides and esters in aqueous solution. Examples of added solutes are alcohols,[45] alkylated ammonium bromides,[46] alkyl sulphates,[47] α-amino acids and derivatives,[48] and N-alkylpyrrolidinones.[49] Generally, hydration of the polar group extends to the third carbon atom in the apolar alkyl tail of solute molecules. A molecular dynamic study of the hydration of methylammonium and acetate ions in aqueous solution confirmed an increase in polar character of the methylene moieties attached to ionic groups with respect to e.g. methane.[50] Moreover, water molecules in apolar hydration shells were not found to be more structured compared to those in the bulk. A study on the dynamics of water molecules in the hydration shell of halogen anions showed that the formation and disruption of hydrogen bonds of water molecules in the solvation shell is slower than that of bulk water.[51]

The presence of hydrophilic functionalities does not prevent the apolar groups from interactions with other apolar moieties. Moreover, interactions of apolar solutes in aqueous solution are strongly favored by electrostatic interactions between oppositely-charged ionic substituents on both components. Some examples were presented in section 1.1.3.

1.2 Aggregation behavior of surfactants

Morphologies of aggregates formed by surfactants in aqueous solution are micelles, vesicles, bicontinuous, or inverted structures;[52] Figure 1.1.

Aggregate morphology is mainly determined by a delicate balance between attractive hydrophobic interactions of surfactant alkyl tails and electrostatic repulsions of surfactant head groups.[53] In addition to repulsive interactions of electrostatic origin, repulsions due to hydration of the head groups must be taken into account. An opposing effect is exerted by the interfacial tension that tends to decrease the effective head group area. The molecular architecture of a given surfactant determines the type of aggregate into which a surfactant associates in aqueous solution. The relationship between the shape of the surfactant monomer and the aggregate morphology can be represented by the packing parameter approach.[54] The packing parameter (P) is calculated from equation (1). In this equation, V is the volume of the hydrocarbon part of the surfactant, l its chain length of the extended all-trans alkyl tail, and a_0 the mean cross-sectional (effective) head group surface area.

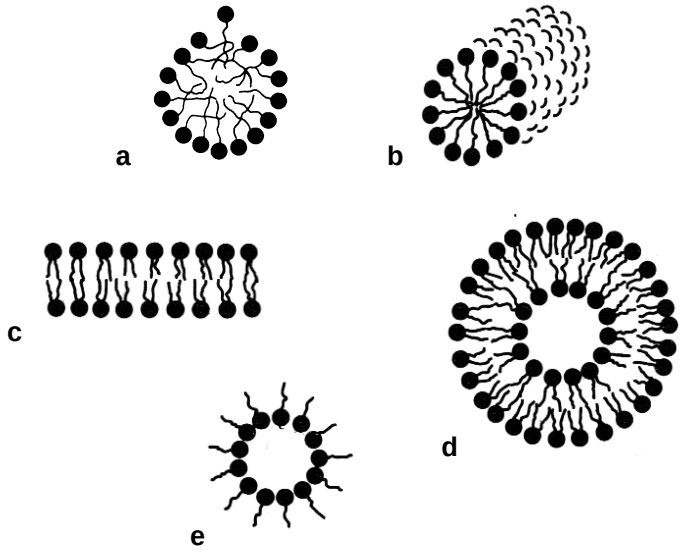


Figure 1.1 Typical aggregate morphologies into which surfactants self-assemble in aqueous solution (a) spherical micelle, (b) wormlike micelle, (c) bilayer fragment, (d) vesicle, and (e) inverted micelle.

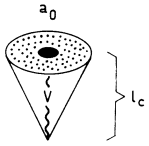
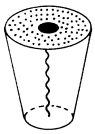
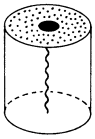
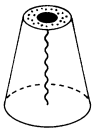
$$P = \frac{V}{a_0 l} \qquad (1)$$

Surfactants where $0<P<1/3$ form micelles in aqueous solution. If $1/3<P<1/2$ wormlike micelles are formed whereas surfactants with $1/2<P<1$ form vesicles. Inverted structures are formed when $P>1$. Table 1.1 shows the relationship of the architecture of surfactant monomer and aggregate morphology.

Several authors have criticized this packing parameter approach for predicting the aggregate morphology.[55,56] For example, sodium dodecylbenzene sulfonate forms micelles in aqueous solution whereas bilayer structures are formed when alkali metal chlorides are added.[56]

Alternatively, it was argued that binding of surfactant monomers to an aggregate and repulsions between surfactant molecules determines aggregate shape.[55]

Table 1.1 Relationship between the shape of surfactant monomers and preferred aggregate morphology.[54]

Effective shape of the surfactant molecule		Packing parameter	Aggregate morphology
	cone	<1/3	spherical micelles
	truncated cone	1/3 – 1/2	wormlike micelles
	cylinder	1/2 – 1	bilayers, vesicles
	inverted (truncated) cone	>1	inverted micelles

1.2.1 Spherical micelles

Generally, unbranched single-tailed surfactants possess a conical shape and aggregate to form spherical micelles in aqueous solution above their critical micelle concentration (cmc).[57] The interior of micelles consisting for the most part of methylene groups of alkyl tails of the surfactants is alkane-like and it contains almost no water. Only the first two methylene groups of the surfactant (counted from the head group) have considerable contact with water in the aqueous solution. The lifetime of a spherical micelle is of the order of milliseconds and the residence time of a monomer in a micelle is of the order of microseconds.[58] Monomer exchange is diffusion controlled. Spherical micelles usually consist of 40-100 monomers[59] and are approximately 5 nm in diameter.

1.2.2 Wormlike micelles

Wormlike micelles are formed by surfactants whose monomer shape resembles a truncated cone. Both theoretical and experimental studies show that wormlike micelles are long (several tens of micrometers) and flexible and that they undergo transformations on relatively short timescales.[60,61,62,63] The presence of wormlike micelles in aqueous solution is often reflected by an increase in relative viscosity.[57] Viscoelastic solutions are formed upon increasing the surfactant concentration; viscoelasticity indicates that an entangled network of wormlike micelles has been formed. Formation of wormlike micelles can often be induced by addition of strongly binding counter ions to ionic surfactants in aqueous solution.

1.2.3 Vesicles

In general, surfactant molecules possessing one head group and two alkyl tails form vesicles in aqueous solution. Actually, upon dissolving double-tailed surfactants in aqueous solution, bilayer fragments are formed that can be closed by the input of mechanical energy.[64] The aggregation and phase behavior of sodium didodecylphosphate (NaDDP) in aqueous solution has been studied in detail.[65] Lamellar phases are formed upon dissolving NaDDP in aqueous solution which transform into myelin figures upon heating the solution above the Krafft temperature. Vesicles are only formed from myelin tubules by stirring.

Vesicles range in diameter from 20 nm to several micrometers and can be either unilamellar or multilamellar. Vesicles formed from pure surfactants are metastable and eventually revert to the flat bilayer state and ultimately precipitate as crystalline materials.

The architecture of a given surfactant monomer is important in determining the morphology of the aggregate into which surfactants self-assemble. Moreover, either addition of certain compounds or changing the solution conditions (e.g. temperature or surfactant concentration) can have a large influence on the aggregate morphology. This subject is discussed in sections 1.2.4 – 1.2.7.

1.2.4 Changing the counter ion

Counter ions have a large influence on the morphology that surfactant aggregates adopt in aqueous solution.[66] Changing the counter ion of ionic surfactants for a more strongly bound one leads to a decrease of the effective head group area. Especially aromatic counter ions like tosylate, benzoate, or salicylate are effective in inducing micellar growth.[66,67,68,69,70,71] In addition to a decrease of a_0 (cf. equation 1), penetration of the aromatic ring of a counter ion between the surfactant molecules leads to an increase in the volume of the surfactant monomer. A combination of both effects results in an increase in P and consequently the surfactants self-assemble into a less curved aggregate. This case corresponds to a change from spherical to wormlike micelles. Upon growing, wormlike micelles may form a three-dimensional network that shows viscoelasticity. The orientation of substituents on the aromatic ring appears to be extremely important for inducing viscoelasticity. For example, hexyltrimethylammonium o-hydroxybenzoate surfactants form a viscoelastic solution

whereas this solution is not formed when the OH substituent is either in the meta or para position.[70,72] Molecular motions of threadlike micelles are fast and the aggregates are dynamic.[73]

1.2.5 Addition of oppositely charged surfactants

Aqueous solutions of cationic and anionic surfactants (catanionic surfactants) may have properties that differ considerably from aqueous solutions of the individual surfactants.[39] For example, aggregate morphologies are usually vesicular at low surfactant concentrations whereas individual surfactants form spherical micelles at low concentration. The effective head group area decreases due to electrostatic interactions between the ionic head groups and due to release of hydration water. The volume of the alkyl tails stays the same. The result is a cylindrical shape for the catanionic surfactant which will consequently self-assemble to form vesicular structures in aqueous solution. A more detailed discussion on catanionic surfactants is given in Chapter 6.

1.2.6 Changing the temperature

Temperature changes can have dramatic effects on the morphology of aggregates formed from nonionic surfactants. The main reason for these changes is the dependence of hydration of nonionic head groups on the temperature. Mixtures of nonionic oligo(ethylene oxide) dodecyl ether ($C_{12}EO_n$) surfactants and phosphatidyl cholines like dioleylphosphatidylcholine (DPPC) undergo a reversible micelle-to-vesicle transition upon changing the temperature.[74,75,76,77,78] The hydration layer of the oxyethylene groups is reduced upon increasing the temperature leading to an decrease of the effective head group area of $C_{12}EO_n$. Moreover, the hydrocarbon chains of DPPC are in more compact cylindrical shape below the gel-to-liquid crystalline phase transition temperatures than above. A combination of both effects leads to a change in the shape of the $C_{12}EO_n$/DPPC surfactant combination from a cone to a cylinder upon increasing the temperature. Similar observations have been made with respect to aqueous mixtures of (nonionic) sugar based surfactants and phosphatidylcholine lipids.[79] In contrast, micelle-to-vesicle transitions in aqueous mixtures of phosphatidylcholine and octyl glucoside occur upon *decreasing* the temperature.[80] Most likely, this results from the temperature dependence of the cmc of octyl glucoside that decreases from 31 mM at 5°C to 16 mM at 40°C. In other words, the membrane solubilizing power of octyl glucoside decreases upon decreasing the temperature.

Although hydration of ionic head groups is less sensitive to temperature changes than head group hydration of nonionics, a_0 (equation 1) of charged head groups slightly increases upon increasing the temperature.[81]

1.2.7 Changing the surfactant concentration

An increase of surfactant concentration in an aqueous solution containing spherical micelles leads to the formation of more micelles.[82] The result is a decrease in the average distance between micelles and an increase in intermolecular repulsions. In order to accommodate the surfactants, spherical micelles may transform into wormlike micelles, which increases the distance between aggregates. The system thus rearranges into aggregates of higher order. Upon further increasing the surfactant concentration, lyotropic liquid crystals can be formed. Two commonly observed liquid crystalline phases are the normal hexagonal phase formed by infinite cylindrical aggregates packed in a two-dimensional array and the lamellar phase.

1.3 Types of surfactants

In addition to conventional single-tailed or double-tailed surfactants (types 1 and 2 in Figure 1.2, respectively) connected to a single head group, other types of surfactants displaying interesting properties have been developed. Figure 1.2 shows schematically different types of amphiphiles.

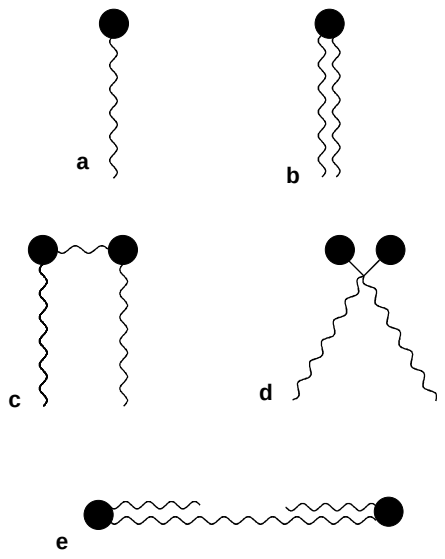


Figure 1.2 Schematic representation of different types of surfactants: (a) single-tailed, (b) double-tailed, (c) and (d) gemini, (e) bolaform.

Gemini surfactants (type c and d in Figure 1.2) are a relatively new type of surfactants although the first gemini had been prepared in the seventies.[83] The name gemini was introduced in 1991 by Menger[84] indicating a surfactant that consists of two hydrocarbon tails each attached to a hydrophilic head group, which are connected by a rigid spacer. Currently, all surfactants containing two hydrophilic head groups, two hydrocarbon tails, and a mirror

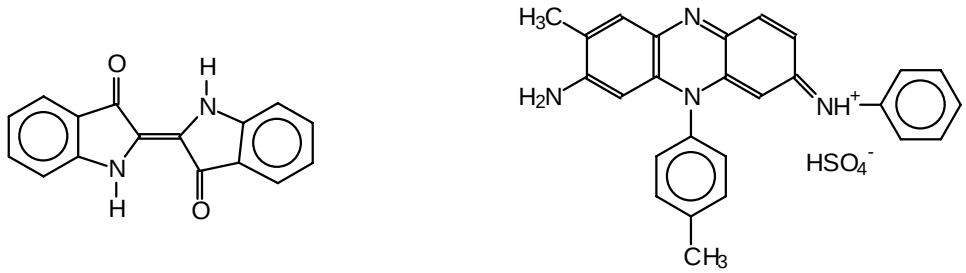
plane or C2 axis are called either geminis or dimeric surfactants even if a spacer is absent (type d in Figure 1.2).[85] Gemini surfactants are superior to conventional surfactants in many ways. For example, geminis display lower critical aggregation concentrations, larger surface tension reduction, lower Krafft temperatures, and better solubility in aqueous solution than their monomeric counterparts.[86] Moreover, dimeric surfactants are superior to many conventional surfactants in oil solubilization.[87,88]

Bolaform surfactants (type e in Figure 1.2) consist of two hydrophilic head groups, connected by a long hydrocarbon spacer.[89,90,91] Aggregation concentrations of bolas are usually lower and aggregation numbers are smaller than those of the monomeric surfactants of which they consist.

1.4 Azo dyes

Dyes have been used since prehistoric times.[92,93,94,95] They were derived from natural products like plants, flowers or berries but also from animal substances. In the 15th century BC the Phoenicians (who lived in the area that is now Lebanon) used an animal dye called tyrian purple, which they obtained from crushed sea snails.[96] Almost 4 million mollusks were required to make one pound of dyestuff. Therefore, only wealthy people could afford to wear clothes colored with this dye. Hence the expression “born to the purple” is often used.[97] The use of the plant dye indigo (Scheme 1.1, left) dates back to the 3rd century BC and it is still an important dye. Indigo-dyed fabrics have been found in Egyptian tombs and in the graves of Incas of Peru. In 1856, William Henry Perkin discovered the first synthetic dye stuff “Mauve” (an oxidation product of aniline, Scheme 1.1 right) while searching for a cure for malaria. This finding initiated a new industry of synthetic dyes and pigments.

Dyes are soluble compounds and possess a specific affinity for the substrates for which they are used. *Pigments*, on the other hand, are insoluble in the media in which they are applied and can only be attached with the help of additional compounds, for example, by using polymers in paints. Dyes are widely used for coloring textiles, leather, in printing, photography. Dyes are also important in new technologies like dye lasers and displays.



Scheme 1.1 Structures of indigo (left) and mauve (right).

Dye molecules consist of chromophores, auxochromes and modifiers. Chromophores are unsaturated groups like aromatic rings, N=N, or C=N moieties that shift the absorption spectrum of the dye from the UV to the visible region. Auxochromes like carboxylate, hydroxide or amino substituents modify the color of the dye and are (if ionizable) responsible for the attachment of dyes to fibers. The color of dyes can also be changed by so-called modifiers: alkyl groups that affect absorption spectra of the dyes.

Azo dyes are an important class of dyes; they are mainly used for dyeing textiles, plastics, leather, paper, mineral oils, and waxes. Their ability to keep an intense color and fastness to light on cellulose fibers is good whereas it is poor on cotton and wool. Azo dyes are classified according to their method of application, to the fibers for which they are used, or their chemical structure. All commercially important azo colorants are listed in the Color Index according to the mechanism of staining, base color, and number.

Azo dyes attach to fibers either covalently or via non-covalent interactions like electrostatic, hydrophobic, or Van der Waals interactions or via hydrogen bonding.

1.4.1 Absorption spectra of azo dyes

The UV-vis absorption spectrum of azobenzene consists of three absorption bands (Figure 1.3).[98] The absorption band corresponding to the lowest transition is at ca. 440 nm (ε ~ 500). This band is assigned to the partly forbidden n→π* transition since it is of low intensity and the band shows a hypsochromic shift upon increasing the solvent polarity. The absorption band centered at 314 nm (ε ~ 17000) is assigned to a π→π* transition. The band shows a bathochromic shift upon increasing the polarity of the solvent and is extremely sensitive to substituent effects. The transition corresponding to the highest energy is localized in the phenyl rings and is due to a π→π* transition. The absorption band occurs at 230-240 nm corresponding to an energy of ca. 500 kJ mol^{-1}. Azobenzenes with an electron withdrawing group on one side of the molecule and an electron donating group on the other side are an example of so-called push-pull molecules which are of interest in the field of non-linear optical materials.[100] The lowest energy π→π*

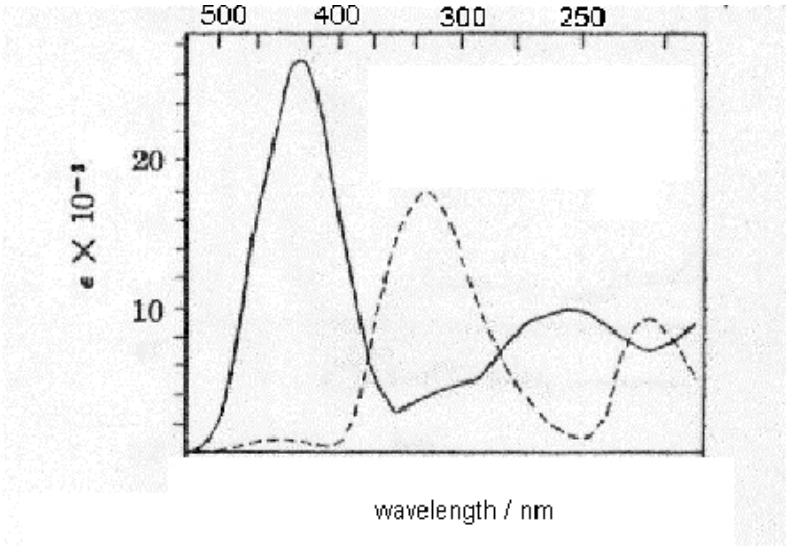
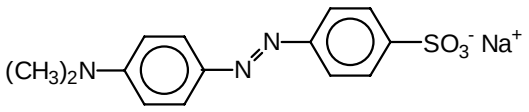


Figure 1.3 Absorption spectra of azobenzene (dotted curve) and 4-dimethylaminoazobenzene (solid curve) in ethanol. Taken from ref. 99.

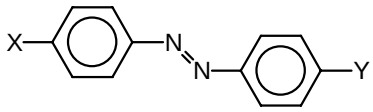
absorption band undergoes a shift to longer wavelengths upon increasing the push-pull character of the substituents on the azobenzene derivative.[101] Table 1.2 presents details of absorption bands of some azo dyes containing different substituents.

Azo dyes like MO (Scheme 1.2) are part of a class of compounds, which are called chromonics.[102] This family of materials also includes drugs, nucleic acids, antibiotics, and carcinogens and anti-cancer agents. Chromonic molecules are disc-like or plank-like, in contrast to the rod-like structure of surfactants. Figure 1.4 shows some examples of chromonic molecules: they usually contain aromatic rings; the hydrophilic groups are arranged at the peripheries of the molecules. Chromonics aggregate by stacking via the hydrophobic sides as a pile of coins or cards rather than by forming micellar structures as is the case for surfactants. As the size of the stacks increases, the fraction of the total hydrophobic surface area exposed to the aqueous subphase decreases although a minimum in the Gibbs energy of aggregation will not be reached and there is no critical aggregation concentration. Aggregation of surfactants into micelles is a cooperative process reflecting a balance between electrostatic repulsions of the ionic head groups and hydrophobic attraction between the alkyl tails.



Scheme 1.2 Structure of MO.

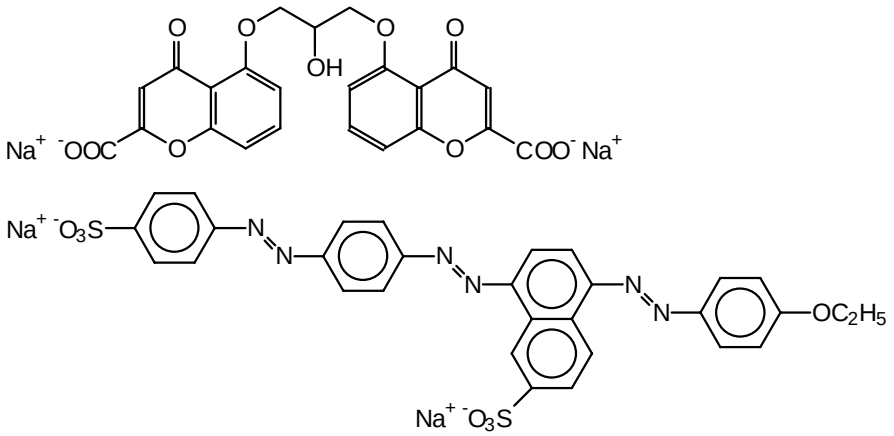
Table 1.2 Absorption bands of azobenzene and its derivatives in ethanol.[95]



X, Y	n→π*		π→π*		π→π*	
	λ (nm)	ε / m² mol⁻¹	λ (nm)	ε / m² mol⁻¹	λ (nm)	ε / m² mol⁻¹
H, H	443	51	319	2200	228	1400
H, OC_2H_5	432	240	349	2260	236	1030
					224	1370
H, $N(CH_3)_2$	[1]	[1]	410	3040	250	1050
NO_2, $N(CH_3)_2$	[1]	[1]	480	1200	280	1260

[1]Overlain by the π→π* band.

Nucleic acid bases and nucleosides aggregate into columnar structures in aqueous solution (Figure 1.5).[102] The standard Gibbs energy increment of aggregation for each additional molecule is nearly constant, irrespective of the column length. Such behavior is called “isodesmic” (Figure 1.6 left). In contrast, surfactant aggregation is characterized by non-isodesmic behavior: micelle formation coincides with a minimum in the Gibbs energy as indicated in Figure 1.6 (right).



disodium cromoglycate (DSCG)[103]

Sirius Supra Brown (RLL)

Figure 1.4 Some examples of chromonic molecules.[102a]

For the aggregation of purine and pyrimidine nucleosides, both the enthalpy and entropy of association are negative.[102a] This indicated that the aggregation process is enthalpically driven in contrast to surfactant aggregation, which is usually entropically driven.[2] The Gibbs energy of aggregation of purine and pyrimidine nucleosides is in the order of kT (ca. 2.5 kJ mol^{-1}).[102a]

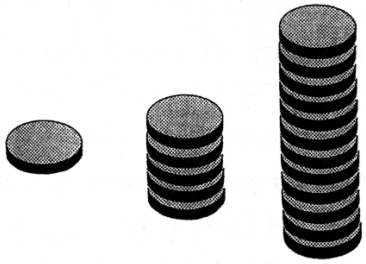


Figure 1.5 Aggregation of chromonic molecules. Taken from ref. 102a.

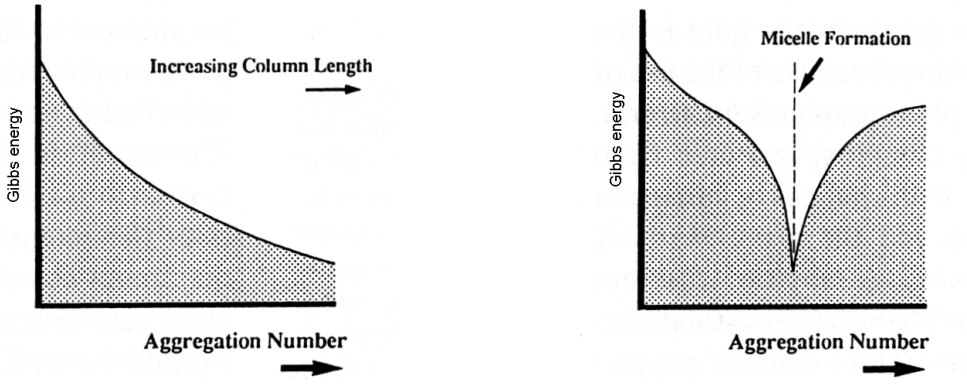
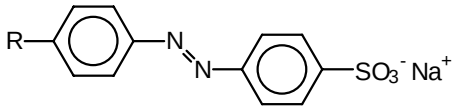


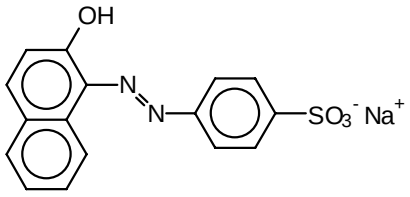
Figure 1.6 Isodesmic behavior displayed by chromonic molecules (left) and non-isodesmic aggregation behavior displayed by surfactants (right). Taken from ref. 91a.

In the case of dyes, aggregation usually occurs only above a certain threshold concentration, similar to aggregation of a surfactant that occurs above the cmc. In a surface tension study on the aggregation of a selection of azo dyes it was shown that a certain

threshold concentration is necessary to induce a lowering of the surface tension in a gradual way.[104] In the series MO to butyl orange (BO) the surface activity increases upon increasing the length of the alkyl substituent from methyl to butyl (Scheme 1.3). Interestingly, ethyl orange (EO) is more surface active than Orange II, which has a larger apolar part than EO. The hydrophobicity of azo dyes was compared to that of n-alkylsulfonates. The nonionic parts of MO, azobenzene sulfonate (ABS), and Orange II are equivalent to a C_6 hydrocarbon tail in the range of 1-10 mM although the lengths of the nonionic parts are about twice that of a C_6 tail.



R	dye
$(CH_3)_2N$	methyl orange (MO)
$(C_2H_5)_2N$	ethyl orange (EO)
$(n\text{-}C_3H_7)_2N$	propyl orange (PO)
$(n\text{-}C_4H_9)_2N$	butyl orange (BO)
H	azobenzene sulfonate (ABS)

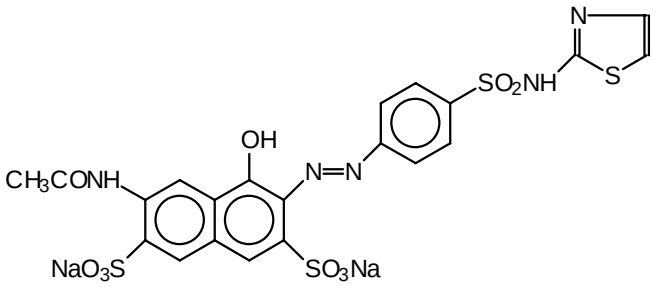


Orange II

Scheme 1.3 Structures of some azo dyes used in a surface tension study.[104]

1.4.2 Dye-protein interactions

The binding of dye molecules to proteins has been studied using spectral techniques. The data provide insight into small molecule-macromolecule interactions, which are of major importance in biochemistry.[105] Klotz et al. extensively studied the interactions of azo dyes and serum albumin[106,107,108,109,110,111] in aqueous solution although other authors[112,113,114] have also investigated this area. Aggregation of MO and bovine serum albumin (BSA) has been investigated spectrophotometrically and by direct binding studies (dialysis). The absorption maximum of MO undergoes a blue shift of ca. 20-30 nm and the intensity of the absorption band decreases by approximately 10% upon addition of BSA. On the other hand, the absorption maximum of azosulfathiazole (Scheme 1.4) shows a red shift from 495 nm in buffer to 505 nm in the presence of 0.2% BSA. Moreover, the intensity of the absorption maximum decreases by ca. 35% (Figure 1.7).



Scheme 1.4 Structure of azosulfathiazole.

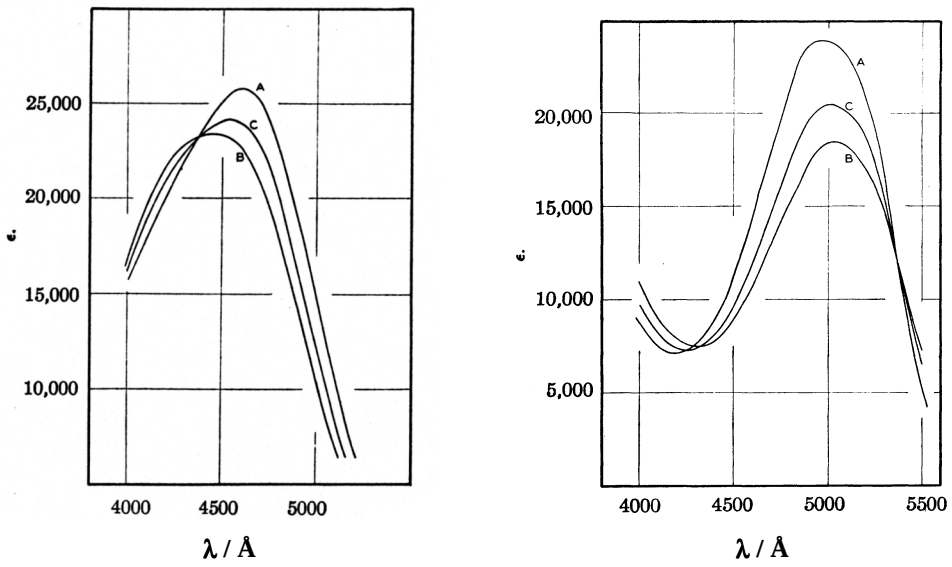


Figure 1.7 Absorption spectra of MO (left) and azosulfathiazole (right) in buffer at pH 7 (curve A), in buffer containing 0.2% BSA at pH 7, (curve B), and in buffer containing potassium phthalate (left curve C) or p-aminobenzoic acid (right, curve C). Taken from ref. 106.

The binding of anions to the protein is assumed to occur via the negatively charged sulfonate moiety of the dyes and positively charged amino acid residues on the protein.[106] Indeed, the maximum number of bound anions per BSA molecule is 22, which roughly corresponds to the 24 arginine residues on the protein that are supposed to be responsible for binding anions. In addition to electrostatic interactions, hydrophobic interactions are thought to play a role in the binding of dyes to proteins. [106,108,109]

The affinity of both BSA and human serum albumin (HSA) increases upon increasing the pH of the aqueous solution.[110] It was proposed that new types of binding sites become available upon increasing pH since the protein undergoes a conformational change upon increasing pH. The absorption maximum of MO bound to HSA shifts to longer wavelengths

upon increasing the pH and is centered at ca. 480 nm at pH 9.2. Since the absorption band resembles that of the dye in acidic solution, it was suggested that the red shift of the absorption band of the dye results from interaction of MO with a proton of an amino acid residue that becomes available at high pH. However, this is unlikely to occur as indicated by a resonance Raman spectroscopy study.[115] Most likely, the red shift can be explained by an increase in the ionic strength of the local environment of the dye due to ionization of additional amino acid residues. The absorption maximum of MO in aqueous solution shifts to longer wavelengths upon addition of either sodium bromide or tetramethylammonium bromide (*vide infra*). In another study the spectral shift of MO to shorter wavelengths upon interaction with proteins was attributed to a twist of the aromatic system since this usually results in a blue shift and in a decrease in the intensity of the absorption band.[113] However, resonance Raman spectroscopy showed that the dye does not undergo a conformational shift upon interaction with proteins.[115]

1.5 Dye-polymer and dye-polysoap interactions

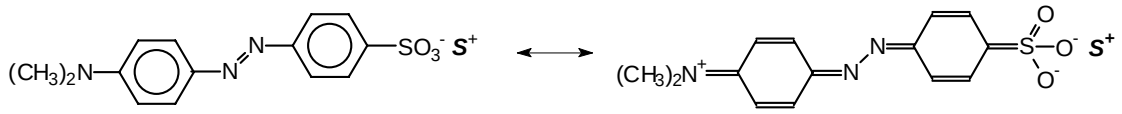
Polysoaps[41,116] and polymers[117,118,119,120] exhibit strong binding towards azo dyes like MO. Both hydrophobic and electrostatic interactions are important in the cooperative binding process.[116,117,118,119] The absorption maximum of the dye undergoes a blue shift from ca. 460 nm in aqueous solution to ca. 370 nm in the presence of small amounts of polymers and polysoaps and it shifts to longer wavelengths upon addition of excess polymer or polysoap. The short wavelength absorption band has been attributed to aggregation of neighboring bound dye molecules on the polymer chain in a parallel orientation.[116,117,118,119] The molecular origin of dye aggregation is discussed in Section 1.7. Upon increasing the polymer or polysoap concentration dilution of the dye molecules over the polymer or polysoap backbone occurs and consequently the absorption maximum shifts to that of monomers. The short wavelength absorption band has also been assigned to a conformational change of the azo group.[113] However, cis-trans isomerism of the azo linkage has been excluded using resonance Raman spectroscopy that showed that MO retains its trans-form upon binding.[115]

1.6 Dye-surfactant interactions

The sensitivity of dyes to the polarity of the medium in which they are dissolved makes them extremely suitable for reporting the presence of hydrophobic microdomains in aqueous solution. Thus, dyes are often used to determine critical micellar concentrations (cmcs) of surfactants. Upon increasing the surfactant concentration, the absorption spectrum of a dye shifts from that in aqueous solution to a spectrum of the dye similar to that in apolar solvents when micelles are present. Frequently used dyes for determining cmcs are for example pinacyanol chloride,[121,122,123] bromophenol blue,[124] and pyrene.[125] Cmcs determined by the dye solubilization method are usually lower than those measured in the absence of dyes.[26] Moreover, the absorption spectrum of the dye often shows anomalities at concentrations far below the surfactants' cmc when the charges of the ionic groups of

surfactant and dye are opposite.[122,126] Therefore it was suggested that, when using charged dyes, the ionic charge should be similar to that of the surfactant.[126]

The observation that interactions occur between oppositely charged surfactants and dyes at concentrations far below the surfactants' cmc is interesting in itself. Several studies of the interaction of dyes and surfactants at low concentration have been reported. MO has been widely studied in this respect.[41,42,43,113,126,127,128] For example, interactions between MO and hexadecyltrimethylammonium bromide (C_{16}TAB) in an aqueous solution of 25 μM of MO occur at a surfactant concentration of approximately 25 μM whereas the cmc of C_{16}TAB is ca. 1 mM.[129] Aggregation is indicated by the appearance of a new band in the absorption spectrum of the dye that is ca. 80 nm blue-shifted with respect to the absorption band in aqueous solution. This band is similar to that of the dye in the presence of small amounts of cationic polymers and polysoaps.[43,113,116] Several explanations have been advanced for the origin of the short wavelength absorption band of MO upon addition of small amounts of cationic surfactants: cis-trans isomerism of the azobenzene moiety,[113] surfactant-dye ion-pair formation,[127] and dye aggregation.[41,42] Cis-trans isomerism of the dye is excluded as described in the previous section.[115] Ion-pair formation of surfactant and dye as shown in Scheme 1.5 is also unlikely since it is not clear why this interaction would only occur at low surfactant concentrations. The dye dissolved in micelles is supposed to interact a similar way with surfactants as shown in Scheme 1.5. Thus, dye aggregation seems the most likely explanation for the short wavelength absorption band in the spectrum of the dyes.



Scheme 1.5 Ion-pair formation was postulated to explain the short wavelength absorption band in the spectrum of MO upon addition of small amounts of cationic surfactants.[127] ***S***$^+$ indicates the surfactant molecule.

1.7 The molecular exciton model

Absorption spectra of dye aggregates usually show large differences when compared to the individual molecules.[130] These differences are caused by so-called exciton coupling between the transition dipole moments of the individual dye molecules.[131] The molecular exciton model qualitatively explains the spectral properties of dye aggregates. The chromophores should preserve their individual characteristics in the aggregates, i.e. it is assumed that there is negligible overlap of respective molecular orbitals. Moreover, the transition moment of the electronic transition is assumed to be localized in the center of the chromophore and its polarization axis parallel to the long axis of the chromophore. Interaction of the excited states of the chromophores in the aggregate leads to a splitting of the excited state, which is give by equation (2).

$$\Delta\nu = \frac{2}{hc}\frac{N-1}{N}\frac{\mu^2}{r^3}(1-3\cos^2\theta) \qquad (2)$$

In this equation, $\Delta\nu$ is the shift of the absorption spectrum for an aggregate consisting of N monomers with respect to the monomer absorption, μ the magnitude of the transition moment, r the center-to-center distance between the chromophores and θ the angle between the chromophore long axes and the chromophore center-to-center line; h is the Planck constant and c the speed of light.

Dye molecules can aggregate either in a parallel (H-aggregation) or in a head-to-tail (J) fashion (Figure 1.8). However, these situations are two extremes since θ can take values from 0 to 90°. The shift from H to J aggregate occurs at θ=54.7° (Figure 1.9).

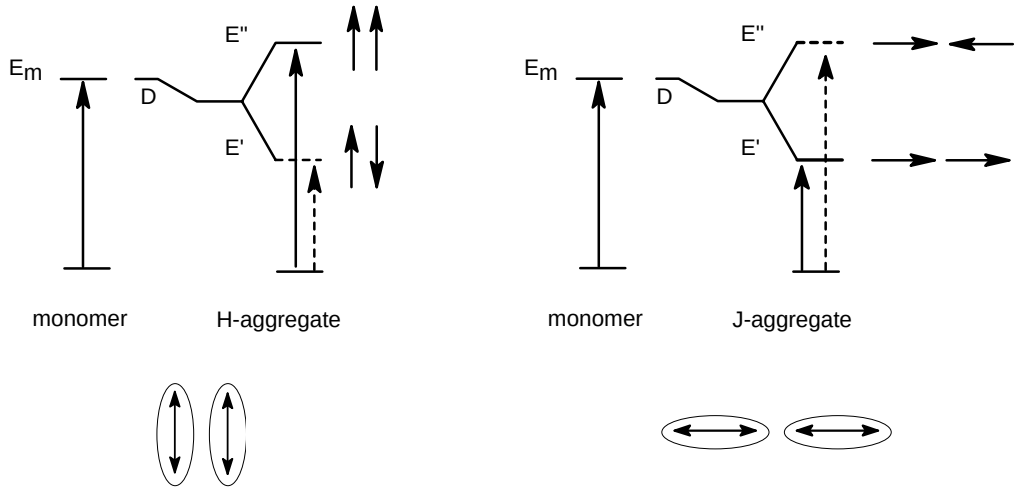


Figure 1.8 Schematic representation of the exciton splitting of the excited state of dye aggregates in a parallel (left) and head-to-tail (right) fashion.

In the case of parallel dye aggregation, transition dipoles can either be aligned in a parallel or in an antiparallel fashion (Fig. 1.8 left). The former situation leads to an excited state that is higher in energy than the excited state in the monomer (due to electrostatic repulsion between the transition dipole moments) whereas antiparallel orientation of the transition dipole moments leads to a decrease in the excited state energy. Since the transition moment of the aggregate is given by the vector sum of both components, transitions from the ground state to E' are forbidden (the transition dipole moments cancel) whereas transitions from the ground state to E'' are allowed. From Figure 1.8 it is clear that the absorption band caused by the dimer consisting of parallel dye dimers will be blue-shifted with respect to that of the monomeric dye. This blue shift can be several tens of nanometers when the separation is 0.5 nm. Another characteristic of H-aggregates is considerable quenching of fluorescence since the transition from the lowest excited state is forbidden in parallel dye aggregates.

When the transition dipoles are in a head-to-tail orientation, the energy level diagram changes to that shown in Figure 1.8 (right). The in-phase arrangement of transition dipoles leads to electrostatic attraction which results in excited state E' whereas out-of-phase arrangement leads to repulsion resulting in state E''. A transition from the ground state to E' leads to a red shift of the transition in the dimer consisting of J-aggregates compared to that of the monomer. Moreover, J-aggregates usually show enhanced fluorescence that is red shifted with respect to the monomer fluorescence.

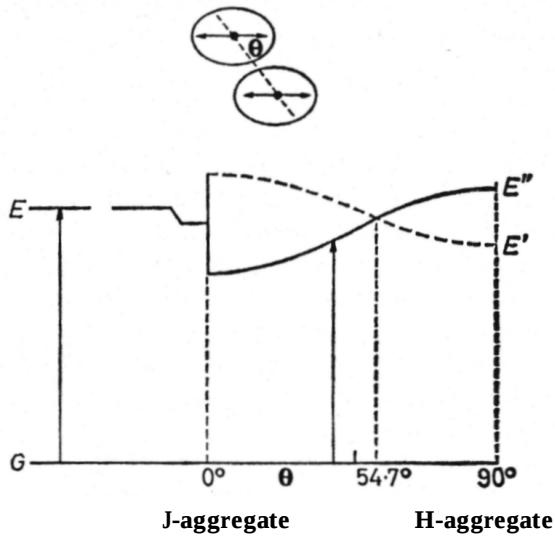


Figure 1.9 Exciton band energy diagram for a molecular dimer with transition dipoles inclined to interconnected axis by angle θ.

1.8 Scope of this thesis

The research reported in this thesis is concerned with the effects of electrostatic interactions on the aggregation of hydrophobic compounds in aqueous solution. As discussed above, a polar functionality attached to an apolar alkyl tail reduces its hydrophobicity by overlap of the hydrophilic and hydrophobic hydration shells. However, aggregation of solutes in two-component systems is greatly facilitated by the presence of ionic groups leading to favorable electrostatic interactions. This effect apparently exceeds the reduction of hydrophobicity of the solutes by the introduction of an ionic group. Destructive overlap of hydrophobic and hydrophilic hydration shells is depicted in Figure 1.10. The effects of electrostatically enhanced hydrophobic interactions are of major importance in many processes in aqueous solution such as protein folding, (surfactant) aggregation, and molecular recognition. However, little is known about factors important in the aggregation process of amphiphilic compounds in aqueous solution governed by both favorable electrostatic and hydrophobic

interactions. The present study was therefore initiated in order to assess their importance in these association processes. Interactions are preferably monitored without the use of probe molecules since the latter might influence aggregation at low concentrations.

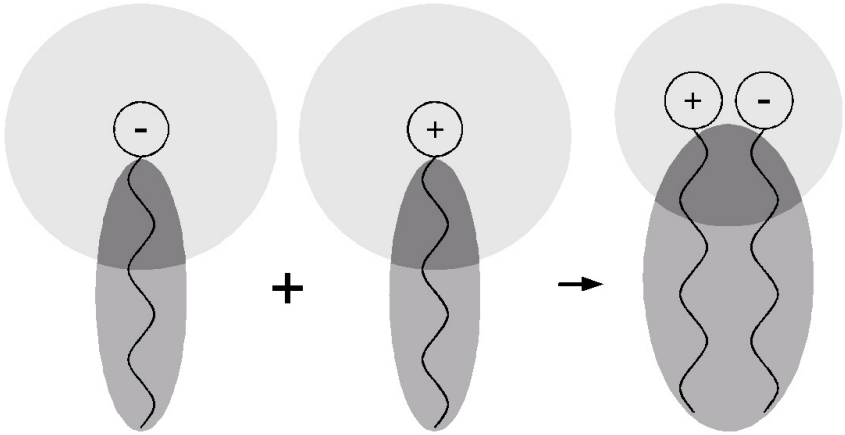


Figure 1.10 Aggregation of amphiphilic molecules leads to hydration shell overlap, a schematic representation.

The initial study was devoted to the interactions of azo dyes and surfactants at low concentrations in aqueous solutions (Chapter 2). Since one of the components contains a chromophore, the association process was directly followed using UV-vis spectroscopy. Aggregation is monitored in the region where both the surfactant and dye do not show interactions individually. Aggregation was studied as a function of the chemical architecture of the oppositely charged compounds. Structural variations in the dye included changes in the type and position of the ionic group in the first aromatic ring, and of the alkyl substituent in the second aromatic ring. The surfactant structure was modified in both the head group region and in the alkyl part. The head groups trimethylammonium and N-methylpyridinium were studied. Moreover, the effects of single-tailed conventional surfactants and dicationic surfactants on the aggregation with azo dyes in aqueous solution were compared. Aggregation occurs at low concentrations and is facilitated by electrostatically favored hydrophobic interactions.

Chapter 3 describes a study of the aggregation of surfactant-azo dye salts in aqueous solution. The counter ion of the surfactants is an azo dye instead of a halide atom. Substitution of the halide counter ion for the hydrophobic azo dye leads to a different morphology of aggregates formed from single-tailed cationic and of dicationic surfactants. Again, an interplay of electrostatic and hydrophobic interactions leads to the observed effects.

Chapter 4 describes the aggregation of n-alkyltrimethylammonium bromide surfactants and methyl octyl orange (MOO). The anionic component contains a larger hydrophobic moiety than the azo dyes studied in the previous two chapters. As a

consequence, the anionic component is a surfactant. Aggregation at low concentrations, where both surfactants do not aggregate individually, were monitored spectroscopically due to the presence of the chromophore in MOO in aqueous solution. Interactions occur at low concentrations and are facilitated by hydrophobic and electrostatic interactions. Aggregate morphologies are studied at higher surfactant concentrations (mM range) and they show a dependence on surfactant alkyl tail length.

Chapter 5 describes a study of the aggregation behavior of n-alkyltrimethylammonium surfactants with a hydrophobically modified salicylate counter ion in aqueous solution. The morphology of aggregates formed from n-alkyltrimethylammonium surfactants is strongly influenced by hydrophobically modified salicylate counter ions as observed by cryo electron microscopy. Temperature induced morphology changes are observed and an explanation is suggested in terms of a vesicle-to-micelle transition.

Chapter 6 describes the aggregation of short-chain compounds ($<C_6$) containing oppositely charged ionic groups in aqueous solution. Intermolecular association processes are studied as a function of the structure of both components, *viz.* alkyl tail length and structure of the ionic group. The influence of hydrogen bonding between the oppositely charged ionic groups is investigated.

Chapter 7 presents a critical review of the investigations described in the previous chapters. The results are discussed in terms of electrostatically-enhanced hydrophobic interactions.

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